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# Phenotypic selection for photosynthetic capacity and efficiency

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## Summary

This work has focused on: 1) Developing new, higher throughput tools for leaf level and canopy level screening, and 2) Screening germplasm sets from CIMMYT (Mexico), UK, and Australia using existing physiological tools such as infrared gas analysis as proxies for variation in Rubisco kinetics and amounts in wheat leaf material in the glasshouse and the field.

## Major Objectives

1. Identification of wheat germplasm with variation in photosynthetic capacity and efficiency under optimal and heat stress conditions.
2. Elucidation of mechanisms underlying improved photosynthesis in candidate lines.
3. Validation of increased photosynthetic performance and yield potential in the field.

## Results and Discussion

### Identification of wheat germplasm with variation in photosynthetic capacity and efficiency: New tools and techniques

A glasshouse pot grown trial was carried out in Canberra in 2012 on 16 genotypes from a panel previously selected for variation in vigour and transpiration efficiency. These genotypes were

grown at limiting and non-limiting nitrogen nutrition to generate a large range of photosynthetic capacity with 3-fold replication. Photosynthetic rates in air and at a range of CO<sub>2</sub> concentrations were measured to allow extraction of Rubisco kinetic parameters by modelling (von Caemmerer and Farquhar 1981), along with specific leaf area, leaf greenness index (SPAD, using 650 and 940nm) and leaf reflectance from 350 to 2500nm using a leaf spectrometer and a modified leaf clip (Fig. 1). A subset of lines was sampled for chlorophyll content and biochemical analysis. Photosynthetic rates in air (A) varied from 6 to 26  $\mu\text{mol m}^{-2} \text{s}^{-1}$ , providing a wide range of photosynthetic performance for validation of new methods.

From reflectance spectra collected from leaves of all genotypes and the other physiological measurements, a partial least squares statistical approach was used to produce a model weighting individual reflectance bands to test the ability of this model to predict photosynthetic parameters in this germplasm set (see Serbin et al. 2012). While the published model for woody species (Serbin et al. 2012) was not suitable for wheat, strong correlations were observed between reflectance features at 380, 540, 706, and 1900 nm, and a robust predictive algorithm for assimilation rate was derived (Fig. 1).

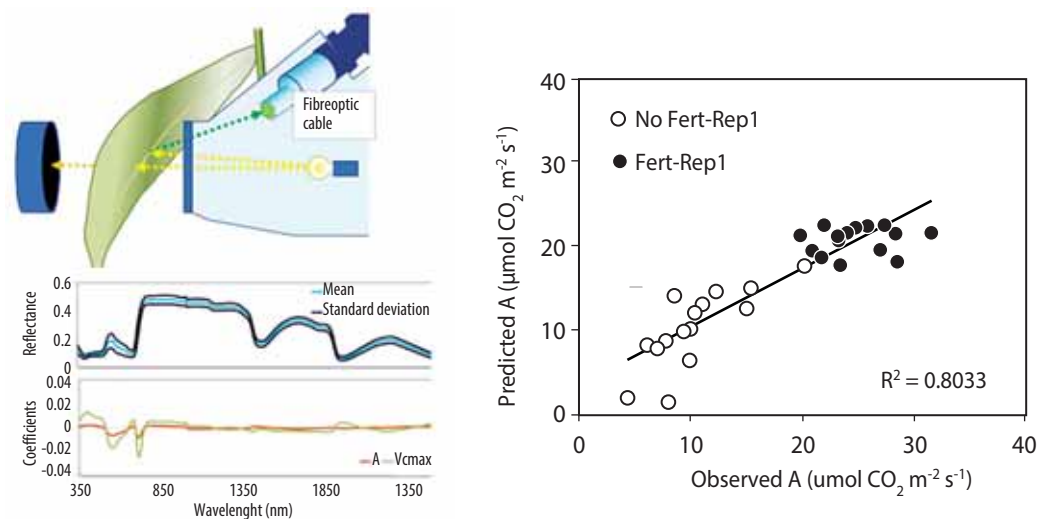


Figure 1. PLS modelling of leaf spectral reflectance for predicting photosynthetic parameters.

SPAD readings correlated with A ( $R^2 = 0.71$ ) and  $V_{cmax}$  ( $R^2 = 0.65$ ). A set of 25 commonly used reflectance indices were calculated from the leaf reflectance data and plotted against photosynthetic parameters. In general, predictive power was poor and NDVI, a reflectance measurement commonly used for crop N estimates, was very poorly correlated with A and  $V_{cmax}$  ( $R^2 < 0.1$ ). Under high fertiliser levels, the predictive capacity of pigment based indices declines due to the saturation of the reflectance signal at high chlorophyll content.

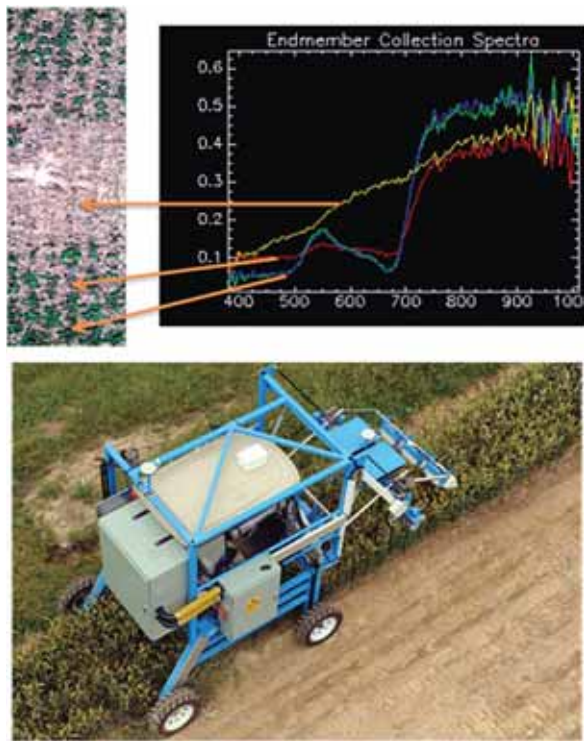


Figure 2. Hyperspectral imaging of a wheat trial pre-canopy closure

A set of 72 diverse genotypes (the **Best Unreleased Yield Potential** or **BUNYIP** set), including Triticale and durum wheat and members of the CIMCOG set, were also grown in the field in August 2012 and similar experiments were performed to test the predictive power of spectral reflectance models at field levels of N nutrition. A subset of 30 of these lines was also grown in single pots in the glasshouse for comparison.

The BUNYIP trial was also measured with a hyperspectral imaging system based on a buggy (the Phenomobile) to test whether these measurements could be scaled to a canopy level (Fig 2).

### Establishing genetic variation in elite wheat germplasm

Photosynthetic characteristics of the CIMCOG population composed by CIMMYT Elite lines were studied in Mexico under controlled conditions (2011, El Batan) and a subset was studied at MEXPLAT (2011-2012, Ciudad Obregon; Fig 3).

Gas exchange measurements were performed at 4 leaf stage under greenhouse conditions and at booting and nine days after anthesis in the field. Genetic variation and genetic range in  $A_{sat}$  and  $g_s$  under both experimental conditions at different growth stages, modeled Rubisco kinetics (maximum carboxylation capacity,  $V_{cmax}$ ) and electron transport rate (ETR) were established for CIMCOG population (Table 1). Genetic variation for chlorophyll content (SPAD), biomass (except seven days after anthesis), radiation use efficiency, and yield were also observed under field conditions (Table 1). Analysis of the data at different growth stages shows 39-50% variation in photosynthetic  $CO_2$



Figure 3. Photosynthesis measurements were performed under greenhouse conditions at 4 leaf stage (A), at booting stage (B) and nine days after anthesis (C) under field conditions.

assimilation rate at ambient CO<sub>2</sub> (Table 1).  $A_{\text{sat}}$  was positively correlated with  $V_{\text{cmax}}$  ( $r=0.72$ ) indicating that  $A_{\text{sat}}$  could be a useful tool to predict Rubisco carboxylation capacity in early leaf stage. ETR was also positively correlated with  $A_{\text{sat}}$  under controlled conditions ( $r=0.68$ ) and could be used as a surrogate to predict photosynthetic carbon assimilation rate in the screening for high photosynthetic rate in large populations (Table 1).

The results shown here indicate that there is genetic variation in the rate of leaf photosynthesis during different growth stages, as previously reported by Evans (1993). The correlation between the rate of leaf photosynthesis and grain yield and biomass at maturity was not observed at initiation of booting ( $r=0.193$  and  $r=0.108$ , respectively), but was observed in the rate measured nine days after anthesis (Figure 4). These results indicate the importance of grain filling photosynthesis to final grain yield and biomass. It is important to consider whether selection for high rates of leaf photosynthesis could

be effective due to spot measurements varying with development stage and other environmental factors (Richards 2000). In this sense it is important to corroborate the present results with additional measurements during 2012-2013 growth cycle, and to complement with additional measurements latter during grain filling.

At Rothamsted, UK, under the BBSRC-funded projects 20:20 Wheat® and CIRC BB/I017372/1, photosynthetic capacity and efficiency was measured for 64 lines in the ERYCC (Earliness & Resilience for Yield in a Changed Climate) panel. A replicated field experiment was set up with the ERYCC panel; phenology was monitored and recorded. At key growth stages, leaf chlorophyll (SPAD) and green canopy cover (spectral reflectance ratios) were measured and leaf area index was estimated. Using fully-emerged flag leaves prior to ear emergence (Zadoks 4.0 – 5.0), the response of photosynthesis to the intercellular CO<sub>2</sub> concentration ( $A/C_i$  curves) and Rubisco

**Table 1. Photosynthetic rate measured at saturating light ( $A_{\text{sat}}$ ), stomatal conductance ( $g_s$ ), maximum carboxylation capacity of Rubisco ( $V_{\text{cmax}}$ ) and electron transport rate (ETR) measured under greenhouse conditions at 4 leaf stage (n=60).  $A_{\text{sat}}$ ,  $g_s$ , SPAD (all measured in flag leaf) and aboveground biomass measured at booting and nine days after anthesis in a selected subset of CIMCOG population (n=30). Biomass was measured seven days after anthesis. Yield and radiation use efficiency (RUE) were measured at maturity also in the same subset of the CIMCOG population (n=30).**

CIMCOG-Green House (2011) and Field Experiment (2011-2012)				
		Mean	P-value (ENT)	Genetic range (LSD 5%)
4 leaf stage	$A_{\text{sat}}$ ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ )	21.4	***	15.1 — 30.4 (5.0)
	$g_s$ ( $\text{mmol H}_2\text{O m}^{-2}\text{s}^{-1}$ )	0.455	***	0.193 — 0.693 (0.153)
	$V_{\text{cmax}}$ ( $\mu\text{molCO}_2\text{m}^{-2} \text{s}^{-1}$ )	122.5	**	94.0 — 155.0 (27.2)
	ETR ( $\mu\text{mol e}^- \text{m}^{-2} \text{s}^{-1}$ )	126.4	***	80.8 — 157.2 (24.9)
Booting	$A_{\text{sat}}$ ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ )	29.9	***	22 – 36 (2.9)
	$g_s$ ( $\text{mmol H}_2\text{O m}^{-2}\text{s}^{-1}$ )	0.459	*	0.263 – 0.624 (0.180)
	SPAD units	47.4	***	40.8 – 55.3 (1.5)
	Biomass ( $\text{g m}^{-2}$ )	667	***	553 – 865 (108)
Anthesis +7/9	$A_{\text{sat}}$ ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ )	23.3	***	15.7 – 27.8 (3.5)
	$g_s$ ( $\text{mmol H}_2\text{O m}^{-2}\text{s}^{-1}$ )	0.349	***	0.183 – 0.610 (0.128)
	SPAD units	52.3	***	46.0 – 57.8 (0.9)
	Biomass ( $\text{g m}^{-2}$ )	1110	ns	970 – 1249 (193)
Maturity	Yield ( $\text{g m}^{-2}$ )	677	***	594 – 797 (44)
	Biomass ( $\text{g m}^{-2}$ )	1464	***	1310 – 1623 (111)
	RUE ( $\text{g MJ}^{-1}$ )	1.69	**	1.33 – 2.10 (0.34)

\* Significant at the 0.05 probability level; \*\* significant at the 0.01 probability level; \*\*\*Significant at the 0.001 probability level.



content were determined for all the genotypes. Preliminary analysis of the data showed a 33% variation in photosynthetic CO<sub>2</sub> assimilation rate at ambient CO<sub>2</sub> (Fig 4) and 25% at saturating CO<sub>2</sub> concentrations (Fig 5). Further data analysis is underway and a second trial has been sown with the same lines.

### Next Steps

Hyperspectral reflectance models will be tested on CIMCOG material at Obregon this 2013 field season. BUNYIP data at the leaf level will be modeled and compared to canopy level measurements. Glasshouse and field trials will be carried out on the CIMCOG set introduced to Australia after passage

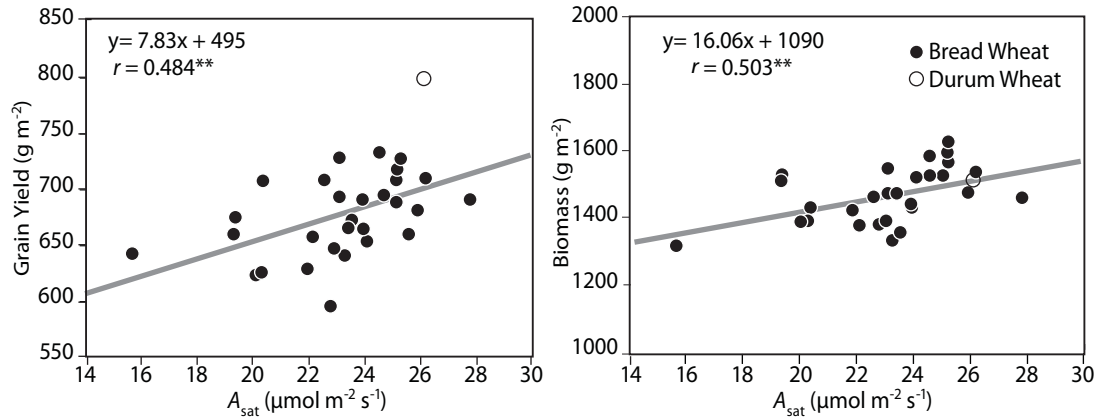


Figure 4. Correlation between grain yield and biomass (at maturity), with photosynthetic rate measured at saturating light ( $A_{sat}$ ) nine days after anthesis in a selected subset of the CIMCOG population (n=30) during the growth cycle 2011-2012.

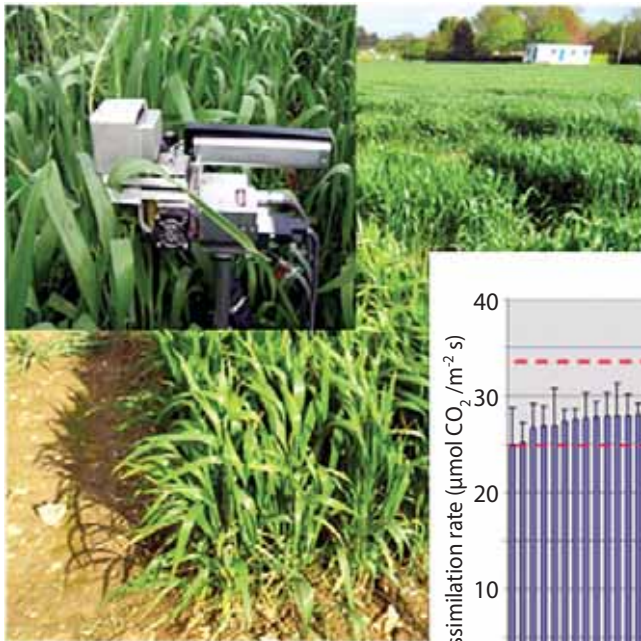
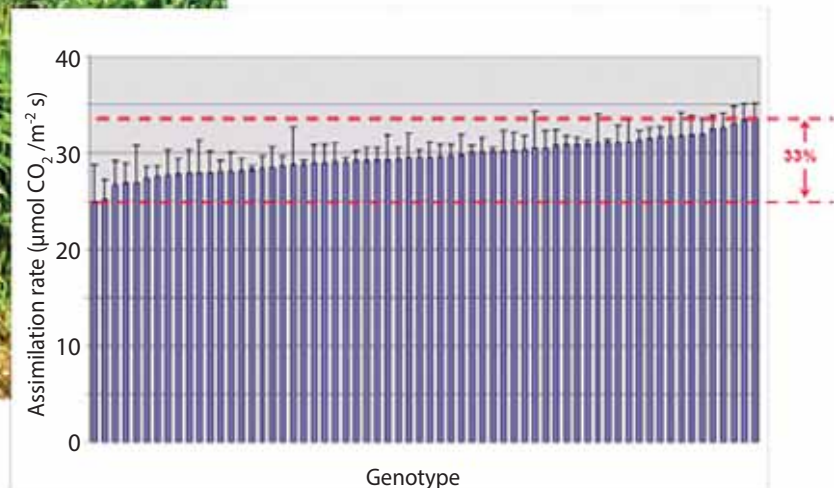


Figure 5. Photosynthetic screening of wheat germplasm at Rothamsted using infrared gas analysis



through quarantine and BUNYIP experiments replicated. Correlations between Rubisco amount and kinetic properties from destructive analysis of leaf material used in the physiological screening in Canberra and UK will be compared with gas exchange, high throughput hyperspectral reflectance, chlorophyll fluorescence, and canopy temperature measurements. Relationships between biomass, harvest index, yield, and photosynthetic characteristics will be collated for all three trial sites.

Measurements of the rate of leaf photosynthesis and at different growth stages together with  $A/C_i$  curves and hyperspectral reflectance measurements will be performed in the subset of CIMCOG population in MEXPLAT during the 2013 cycle in order to (i) determine consistency of genetic variation in photosynthetic related parameters, (ii) determine maximum carboxylation capacity under field conditions, and (iii) establish a model to predict photosynthetic parameters based on hyperspectral reflectance as described above.

Additional to these measurements, leaf photosynthetic rate and  $A/C_i$  curves will be measured in a selected subset of lines from a landrace population selected from the germplasm bank. These lines were selected under field conditions during 2012 for their high Biomass. The objective will be to identify candidate lines with high photosynthetic rate during grain filling and high carboxylation capacity of Rubisco to incorporate these candidate lines in strategic crosses with selected CIMCOG lines.

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